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THE CONSTITUENTS OF HOPS

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CXXXV.—*The Constituents of Hops.*

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On account of the extended use of hops in the brewing industry, and also, but in a very much less degree, for medicinal purposes, they have been the subject of numerous investigations. The chief object of these investigations appears to have been to ascertain the nature of the bitter principles, or to determine the proportion in which they are present. Although a considerable number of products have thus from time to time been obtained, to which various names and formulæ have been assigned, the character of most of them clearly indicates that they do not represent definite chemical compounds. Apart from the essential oil yielded by the distillation of hops with steam, which has been the subject of several independent investigations by Chapman and others (T., 1903, **83**, 505; *J. pr. Chem.*, 1911, [ii], **83**, 483), the determination of the presence of tannin and sugar, and the isolation of a very small amount of choline, the information respecting the other constituents, which are mostly contained in the resinous material, has been exceedingly deficient and by no means satisfactory. The confusion which pervades the subject will be apparent from the following brief survey of the literature.

Vlaanderen (*Jahresb. Chem.*, 1858, p. 448) obtained from lupulin (the glandular powder from hops) a resin to which he assigned the formula $C_{54}H_{70}O_{11}$, and from which copper compounds of varying composition were prepared.

Wagner (*Dingler's polyt. J.*, 1859, **154**, 65), in determining the tannin of hops, states that he found a yellow colouring matter which behaved towards reagents like quercitrin, and could be split into quercetin and dextrose, but no evidence of the identity of these substances was recorded. The tannin ("humulotannic acid") has been especially studied by Etti (*Annalen*, 1876, **180**, 223), and more recently by Chapman (*J. Inst. Brewing*, 1907, **13**, 646), with reference to its quantitative determination.

Lermer (*J. pr. Chem.*, 1863, **90**, 254) states to have isolated from hops a crystalline substance, insoluble in water, but the alcoholic solution of which possessed a bitter taste. This substance, which was very unstable, becoming in a few hours soft and yellow, was called the bitter acid of hops ("hopfenbittersäure"), and from the analysis of a copper compound it was considered to possess the formula $C_{32}H_{50}O_7$. Lermer also indicated that the hop gland contains a wax consisting of myricyl palmitate.

H. Bungener (*Bull. Soc. chim.*, 1886, [ii], **45**, 487) confirmed the observation of Lermer (*loc. cit.*) respecting the occurrence of a crystalline acid, insoluble in water, which rapidly became yellow and resinous on exposure to the air. He designated the substance "lupulic acid," stating it to melt at 92—93°, and to agree in composition with the formula $C_{50}H_{70}O_8$. It was furthermore considered that it is the resinous oxidation product of this acid which imparts bitterness to a decoction of hops, and that this resinous product exists in hops together with the acid from which it is readily formed. Bungener and Fries (*Zeitsch. ges. Brauw.*, 1885, **8**, 267) have stated that hops contain asparagine, but although the method of separation was given, no analysis or other evidence of the identity of the substance appears to have been recorded.

Issleib (*Arch. Pharm.*, 1880, **13**, 345), from the results of a somewhat extended investigation, has concluded that hops contain a peculiar bitter substance, "hop-bitter," of the composition $C_{29}H_{46}O_{10}$. This, when treated with dilute sulphuric acid, is said to be converted into a resinous product, "lupuliretin," $C_{10}H_{16}O_4$, and a crystalline, bitter substance, "lupulinic acid,," $C_{48}H_{82}O_{19}$, which is insoluble in water, the change being represented by the following equation:

$$2C_{29}H_{46}O_{10} + 3H_2O = C_{10}H_{16}O_4 + C_{48}H_{82}O_{19}.$$

The same author, furthermore, assigns to hop-resin the formula $C_{10}H_{14}O_3$, and assumes that by the simple addition of a molecule of water it is converted into "lupuliretin," $C_{10}H_{16}O_4$. On the other hand, hop-resin, $C_{10}H_{14}O_3$, is considered to be produced by the oxidation of the essential oil of hops, the latter being given the formula $C_{10}H_{18}O$, and the change represented as follows: $C_{10}H_{18}O + 2O_2 = C_{10}H_{14}O_3 + 2H_2O$. Inasmuch as it is well known, however, that both hop-resin and the essential oil of hops are very complex products, the latter consisting largely of a sesquiterpene, $C_{15}H_{24}$, the fallacy of the above conclusions is apparent.

Hayduck (*Woch. Brau.*, 1887, **4**, 397; 1888, **5**, 937) has differentiated three resinous constituents of hops as follows: α-resin, which is soft, soluble in light petroleum, and precipitable by an alcoholic solution of lead acetate; β-resin, which is similar to the preceding, but is not precipitable by lead acetate; and γ-resin, which is a hard solid, insoluble in light petroleum, but soluble in ether or alcohol, and not precipitable by lead acetate. The solutions of both the α-resin and the β-resin were said to be intensely and disagreeably bitter.

Lintner and A. Bungener (*Zeitsch. ges. Brauw.*,1891, **14**, 357) note that in the α-resin of Hayduck (*loc. cit.*) crystals were formed after some time which appeared to be different from H. Bungener's hop-

bitter acid (*loc. cit.*). They obtained a crystalline product, melting at 56°, which showed but little tendency to resinify, and this was supposed to be identical with the bitter substance of Lermer. Seyffert and von Antropoff (*Zeitsch. ges. Brauw.*, 1896, **19**, 1), in an attempt to compare the products obtained by them with those of other investigators, were led to conclude that hop resins are mixtures of substances in a state of progressive decomposition.

Barth (*Zeitsch. ges. Brauw.*, 1900, **23**, 509, 537, 554, 572, 594), who has reviewed the results of earlier investigators, states that the formula of lupulic acid is not $C_{50}H_{70}O_8$, as given by H. Bungener (*loc. cit.*), but $C_{25}H_{36}O_4$, and that there is no doubt of its identity with Lermer's bitter acid. On oxidation with permanganate, it gave valeric acid. He also indicates that the so-called lupulic acid does not contain a carboxyl group, since it can be recovered from an alkaline solution by extraction with ether. On the other hand, it is stated that when fused with potassium hydroxide, it yields a hydrocarbon having a density of 0·8840 at 15°, the composition of which is most simply expressed by the formula C_5H_8. From this result it was considered that lupulic acid is a derivative of an olefinic terpene. The author, furthermore, notes the points of distinction between α-lupulic acid (m. p. 54—56°), which was observed by Hayduck to be a constituent of the so-called α-resin, and is said to crystallise in small rhombohedra, and β-lupulic acid (m. p. 92°), which has been described as forming long, glistening prisms. Both compounds were presumed to be unsaturated, to possess similar molecular weights, and to stand in some relationship to the essential oil of hops.

Bamberger and Landsiedl (*Zeitsch. ges. Brauw.*, 1902, **25**, 461) describe the α-lupulic acid or hop-bitter acid as an amber-coloured, crystalline mass (m. p. 56°), which is readily soluble in nearly all solvents, and when oxidised by exposure to the air gives with ferric chloride a yellowish-brown coloration. They considered it to possess the formula $C_{20}H_{28}O_5$ or $C_{20}H_{30}O_5$.

Lintner and Schnell (*Zeitsch. ges. Brauw.*, 1904, **27**, 666) were not successful in their attempts to obtain α-lupulic acid in a pure state, and they therefore examined the products formed from the acid by the action of an alcoholic solution of sodium hydroxide. They thus obtained, besides resinous matter and valeric acid, a crystalline substance (m. p. 92·5°), which was considered to possess the formula $C_{15}H_{24}O_4$, and to be an unsaturated hydroxyketonic acid. They also proposed to designate the above-mentioned α-acid as "humulon," and its crystalline product of decomposition as "humulin," retaining for the so-called β-acid the name "lupulic acid."

Siller (*Zeitsch. Nahr. Genussm.*, 1909, **18**, 241), with consideration of the investigation of Hayduck (*loc. cit.*), has proposed a method for quantitatively determining the α-, β-, and γ-resins of the last-mentioned author, and also the so-called α-hop-bitter acid. Since the latter substance was obtained from the lead compound of the mixed resins by decomposing it with sulphuric acid, it must have consisted to a large extent of ordinary fatty acids, which the present authors have shown to be contained in considerable amount in hops.

Adler (*Zeitsch. ges. Brauw.*, 1912, **35**, 406) and Seibriger (*Woch. Brau.*, 1913, **30**, 177, 196) have suggested methods for determining the bitter substances of hops by extracting the latter with light petroleum or carbon tetrachloride, and titrating the resulting extract with alcoholic potassium hydroxide. Such methods, however, are entirely empirical, and, as in the case of the method proposed by Siller (*loc. cit.*), the substances actually determined would no doubt consist chiefly of fatty acids.

Griessmayer (*Dingler's polyt. J.*, 1874, **212**, 67) distilled a decoction of hops with an alkali hydroxide, and obtained, besides ammonia and trimethylamine, a small amount of a liquid which was said to have an odour resembling that of coniine. This very indefinite substance was presumed to be a volatile alkaloid, for which the name "lupuline" was proposed, but no crystalline salt could be prepared from it, nor does the presence of nitrogen appear to have been determined.

Griess and Harrow (*Ber.*, 1885, **18**, 717) showed that hops contain a very small proportion (not more than 0·02 per cent.) of choline. As a very dilute solution of choline was found to dissolve relatively large amounts of hop-resin, whereby an intensely bitter taste was imparted to the liquid, they thought it not unlikely that such a combination occurs in the hops, thus forming the bitter substance which is soluble in water.

The preceding survey of the very voluminous literature pertaining to the chemistry of hops, although by no means complete, will afford an indication of the present state of knowledge on the subject. In view of the existing confusion, it has been considered that other and more systematic methods of examination than those hitherto employed might serve more clearly to define the character of the constituents of this important natural product, and thus explain the numerous discrepancies of statement. The results of the present investigation, with the deductions from them, are summarised at the end of this paper.

Experimental.

The material used in this investigation consisted of a good quality of fresh Kentish hops, from the crop of 1911.

A small portion (10 grams) of the material was subjected to a preliminary test for an alkaloid, when indcations were obtained of the presence of a very small amount of such a substance.

In order to ascertain whether an enzyme were present, 200 grams of the ground material were macerated with water at the ordinary temperature. To the expressed and filtered liquid about twice its volume of alcohol was added, but the precipitate thus produced was quite insignificant.

Determinations of the moisture and ash, with the use of air-dried material, gave the following results:

0·5762 of hops, on heating at 110°, lost 0·0604 H_2O. $H_2O = 10·48$ per cent.

10·0 of hops gave, on incineration, 0·7919 of ash. Ash = 7·919 per cent.

The amount of tannin present was determined by Chapman's method (*J. Inst. Brewing*, 1907, **13**, 646) with the following results:

Two portions of infusion, each representing 1 gram of hops, gave 0·0400 and 0·0402 respectively of cinchonine tannate. The corresponding amounts of tannin are thus equivalent to 2·40 and 2·41 per cent. respectively of the weight of the hops.

A portion (25 grams) of the ground hops was completely extracted in a Soxhlet apparatus with various solvents, when the following amounts of extract, dred at 100°, were obtained:

Petroleum (b. p. 35—50°)	extracted	5·45	grams	= 21·80	per	cent.
Ether	,,	0·65	,,	2·60	,,	,,
Chloroform	,,	0·60	,,	2·40	,,	,,
Alcohol	,,	2·15	,,	8·60	,,	,,
Total.....		8·85	grams	35·40	per	cent.

For the purpose of a complete examination, 25 kilograms of the ground material were completely extracted with hot alcohol, when, after the removal of the greater part of the solvent, 9·1 kilograms of a viscid, dark green extract were obtained.

Distillation of the Extract with Steam and Separation of the Essential Oil.

The entire amount of the above-mentioned extract was mixed with water, and the mixture distilled in a current of steam. On extracting the distillate with ether, and removing the solvent, a quantity of a pale yellow, essential oil was obtained. This oil

naturally differed considerably in its characters from that obtained by the direct distillation of hops with steam, and, with consideration of the fact that it is not a normal product, it has not been deemed desirable to record its constants.

Non-volatile Constituents of the Extract.

After the removal of the essential oil, as above described, there remained in the distillation flask a dark-coloured aqueous liquid (*A*) and a dark green, oily resin (*B*). The resin was separated, and, in order to wash it, thoroughly mixed with boiling water, when an emulsion was formed. The latter was shaken with light petroleum, after which the aqueous layer, which contained suspended resin, was filtered, the filtrate being concentrated and added to the main portion of the aqueous liquid. The petroleum liquid was then evaporated, and the residue mixed with the solid which had been collected on the filter, the total amount of resin being thus brought together.

Examination of the Aqueous Liquid (A).

A portion of the aqueous liquid, representing 2 kilograms of the original extract, was first thoroughly extracted with ether and then with chloroform. These solvents removed some brown, viscid material, amounting to about 12 and 3 grams respectively. Both of these products possessed a very bitter taste, and gave a brown coloration with ferric chloride. Their respective solutions in ether and chloroform were shaken with dilute aqueous hydrogen chloride, but the acid liquids afforded no definite indications of the presence of an alkaloid. On subsequently extracting the ethereal and chloroform liquids with aqueous alkalis, only dark-coloured, amorphous products were obtained.

The aqueous liquid which had been extracted with both ether and chloroform was next shaken with several successive portions of amyl alcohol. This treatment removed a quantity (about 29 grams) of a dark brown, viscid product, which possessed an acrid taste and yielded, in aqueous solution, a green colour with ferric chloride. Separate portions of it were heated with dilute sulphuric acid and aqueous potassium hydroxide respectively, but nothing definite could be obtained by this treatment.

After extracting the aqueous liquid with amyl alcohol, as above described, it was concentrated somewhat under diminished pressure and then treated with a slight excess of basic lead acetate solution, when a brown precipitate was produced. The latter was collected, washed, suspended in water, decomposed by hydrogen sulphide, and the liquid filtered. The filtrate, on concentration under diminished

pressure, yielded a dark-coloured product which appeared to consist only of tannin and amorphous colouring matter. This conclusion was confirmed by heating a portion of the liquid with potassium hydroxide, acidifying, extracting with ether, and shaking the ethereal liquid with aqueous ammonium carbonate. A small amount of substance was thus obtained, which evidently consisted of gallic acid, and had been formed from the tannin originally present. On methylation, it yielded trimethylgallic acid, melting at 164°.

The filtrate from the precipitate produced by basic lead acetate was deprived of lead by means of hydrogen sulphide, filtered, and concentrated to the consistency of a syrup. The latter evidently contained a large quantity of sugar, since it readily yielded *d*-phenylglucosazone (m. p. 208—209°), but it gave nothing crystalline on acetylation. When diluted with alcohol, and kept for some time, it deposited a quantity (about 5·5 grams) of potassium nitrate.

Isolation of Choline, $C_5H_{15}O_2N$.

The occurrence of choline in hops was conclusively shown some years ago by Griess and Harrow (*Ber.*, 1885, **18**, 717), but for the purpose of completeness it was deemed desirable to determine its presence in the material under investigation. The above-mentioned liquid, from which the potassium nitrate had been removed, was therefore concentrated under diminished pressure, the syrupy liquid thoroughly extracted with alcohol, and the resulting liquid evaporated. The residue from the latter was repeatedly treated with alcohol in the same manner until a product was finally obtained which was soluble in nearly absolute alcohol. To the alcoholic solution thus obtained, a little hydrochloric acid was added, and subsequently an alcoholic solution of mercuric chloride, the mixture being then kept for some time. The precipitate which had formed was collected, dissolved as completely as possible in warm water, and the mercury removed from the solution by means of hydrogen sulphide, after which the filtered liquid was nearly neutralised with sodium carbonate and evaporated to dryness under diminished pressure. The product was then treated with absolute alcohol, the filtered liquid concentrated, and this operation repeated until the inorganic salt was completely eliminated. On the addition of platinic chloride to the final alcoholic solution, an orange-coloured precipitate was produced. The latter was collected, washed with absolute alcohol, and dried on a porous plate, when it melted and decomposed at 233—238°:

0·0304, dried at 110°, gave 0·0098 Pt. Pt = 32·2.

$(C_5H_{14}ONCl)_2PtCl_4$ requires Pt = 31·7 per cent.

Isolation of l-*Asparagine*, $C_4H_8O_3N_2$.

Although it has been stated by Bungener and Fries (*Zeitsch. ges. Brauw.*, 1885, **8**, 267), that hops contain asparagine, they appear to have recorded no analysis or other evidence of the identity of the substance isolated, and it therefore seemed desirable to confirm its presence.

An amount of the original aqueous liquid (*A*), representing one kilogram of the alcoholic extract of the hops, was treated with a slight excess of barium hydroxide, and the excess of alkali then removed by means of carbon dioxide. To the filtered liquid a slight excess of a solution of basic lead acetate was added, and the filtrate from the precipitate thus produced then slightly acidified with acetic acid. On the subsequent addition of an aqueous solution of mercuric nitrate, prepared with the aid of just sufficient nitric acid to dissolve the salt, a considerable amount of a nearly white precipitate was produced. This was collected, washed, suspended in water, and decomposed with hydrogen sulphide, after which the filtered liquid was neutralised with ammonia, then slightly acidified with acetic acid, and concentrated under diminished pressure. On keeping the resulting syrup, a quantity (4·2 grams) of a substance separated in large, colourless prisms, which were recrystallised from water:

0·1972 * gave 0·2635 CO_2 and 0·1095 H_2O. C = 36·4; H = 6·2.
$C_4H_8O_3N_2$ requires C = 36·4; H = 6·1 per cent.

This substance was lævorotatory in aqueous solution, and was thus evidently *l*-asparagine. The amount obtained was equivalent to about 0·015 per cent. of the weight of hops employed.

Examination for a Volatile Alkaloid.

It was noted many years ago by Griessmayer (*Dingler's polyt. J.*, 1874, **212**, 67) that hops contain a very small amount of a volatile base, which possesses an odour resembling that of coniine, and it was therefore sought to confirm this statement.

A quantity of the aqueous liquid (*A*), representing 4 kilograms of the original alcoholic extract of the hops, was concentrated, rendered alkaline with potassium hydroxide, and distilled in a current of steam for four hours. The distillate was then extracted with ether, the ethereal liquid being subsequently shaken with dilute hydrochloric acid. This aqueous acid liquid was made alkaline with potassium hydroxide, extracted with ether, and the ethereal liquid evaporated, when a small residue, having a strong,

* Dried at 130°.

coniine-like odour, was obtained. The residue was dissolved in a small amount of dilute alcohol, acidified by the addition of a few drops of decinormal hydrochloric acid, the liquid filtered, and evaporated to dryness in a vacuum desiccator. A very small, varnish-like residue was thus obtained, which, when dissolved in water, gave decided reactions with the usual alkaloid reagents, and when made alkaline developed an odour recalling that of coniine. This result served to confirm the observation of Griessmayer (*loc. cit.*) respecting the occurrence in hops of a volatile alkaloid, but the amount present is so extremely small that it has not been possible further to characterise it.

Examination of the Resin (B).

The crude, resinous material which had been separated from the aqueous liquid (*A*), as previously described, was mixed with purified sawdust, and the thoroughly dried mixture extracted successively in a large Soxhlet apparatus with various solvents. The weights of the products, as determined by evaporating aliquot portions of the respective extracts and drying the residues at 100°, were as follows:

Petroleum (b.p. 35—50°)	extracted	2635	grams.
Ether	,, ,,	692	,,
Chloroform	,, ,,	66	,,
Ethyl acetate	,, ,,	30	,,
Alcohol	,, ,,	104	,,
Total...		3527	grams

The above amount of resin is equivalent to about 14·1 per cent. of the weight of hops employed.

Petroleum Extract of the Resin.

This extract, after the complete removal of the solvent, formed a dark green, viscid mass. A quantity (750 grams) of the extract was introduced into a distillation flask, some water added, and steam passed through the mixture for five hours. By this means a small amount of essential oil was eliminated which had not been removed by the distillation of the original alcoholic extract with steam. The contents of the distillation flask were then shaken with a large volume of ether, and the ethereal layer, which contained a relatively small amount of a sparingly soluble solid in suspension, was filtered, the solid being well washed with ether. This solid was a neutral, wax-like substance, and could not be obtained free from chlorophyll by crystallisation. It was therefore heated with alcoholic potassium hydroxide, when it yielded ceryl alcohol (m. p. 78°) and cerotic acid (m. p. 79°), and thus appears

to have consisted of ceryl cerotate. Larger amounts of both the respective alcohol and acid were subsequently obtained. The ethereal filtrate and washings from the wax, which were free from alkaloid, appeared to contain chiefly fatty matter and chlorophyll, together with some free fatty acids. As it was not deemed necessary to examine the free and combined fatty acids separately, the ether was removed, the residue dissolved in alcohol, and hydrolysed by boiling for two hours with potassium hydroxide, when during the operation ammonia was evolved. The alcohol was then removed, water added, and steam passed through the mixture for some time. By this treatment the last remaining portions of essential oil occluded by the fatty material were eliminated, and, on examination, were found to consist chiefly of sesquiterpene.

The alkaline, aqueous liquid which had been distilled with steam, as above described, was extracted many times with ether for the removal of the unsaponifiable material. During this operation an emulsion formed in the lower portion of the ethereal layer, and this, when collected and well washed with ether, yielded a soap-like solid, which was found to consist of potassium cerotate.

Examination of the Unsaponifiable Material.

The ethereal liquid obtained by extracting the hydrolysed petroleum extract of the resin with ether, as above described, was dried, and the solvent removed. A viscid, dark yellowish-brown product was thus obtained, which amounted to about 140 grams.

Isolation of Ceryl Alcohol and Hentriacontane.

The above-mentioned, dark yellowish-brown product was dissolved in about 700 c.c. of hot alcohol, when, on cooling, a solid separated and was collected. This solid, which was obviously a mixture, was dissolved in pyridine, and heated for a short time with an excess of phthalic anhydride. Water was then gradually added to the warm liquid, and the mixture extracted with ether, the ethereal liquid being freed from pyridine by means of dilute sulphuric acid, and then shaken with aqueous sodium carbonate. During the latter operation a quantity of a solid sodium salt of an acid phthalic ester separated, and was collected. The latter was hydrolysed with alcoholic potassium hydroxide, after which the alcohol was removed, water added, and the mixture extracted with ether. A quantity of a solid was thus obtained which, when crystallised from alcohol, separated in colourless, glistening leaflets, melting at 78—79°. This substance, which amounted to 5 grams, was identified as ceryl alcohol (Found, C=81·7; H=14·0. Calc., C=81·8; H=14·1 per cent.).

The ethereal liquid from which the sodium salt of the acid phthalic ester of ceryl alcohol had been separated, as above described, was dried and evaporated, when a solid (about 3 grams) of low melting point was obtained. The latter was crystallised from ethyl acetate, when it formed nacreous leaflets, melting sharply at 68°, and was identified as hentriacontane (Found, C = 84·8; H = 14·9. Calc., C = 85·3; H = 14·7 per cent.).

Isolation of a Phytosterol, $C_{27}H_{46}O$.

The original alcoholic mother liquors from the mixture of ceryl alcohol and hentriacontane were diluted somewhat with water, and kept for some time, when, although the greater part of the material separated as a brown, viscid product, a substance in the form of crystalline plates was slowly deposited. The latter substance, which gave the colour reaction of the phytosterols, was collected, and crystallised several times from a mixture of ethyl acetate and dilute alcohol. It then separated in large, colourless leaflets, melting at 135—136°, and amounted to about 2 grams:

0·1164, on heating at 110°, lost 0·0054 H_2O. $H_2O = 4·6$.

0·1110 * gave 0·3402 CO_2 and 0·1194 H_2O. C = 83·6; H = 12·0.

$C_{27}H_{46}O,H_2O$ requires $H_2O = 4·5$ per cent.

$C_{27}H_{46}O$ requires C = 83·9; H = 11·9 per cent.

0·3552,* made up to 20 c.c. with chloroform, gave α_D −1°6′ in a 2-dcm. tube, whence $[\alpha]_D$ −30·9°.

The above-described substance was thus identified as a phytosterol. It yielded an acetyl derivative which separated from ethyl acetate in flat needles, melting at 121—122°.

Examination of the Volatile Fatty Acids.

The alkaline, aqueous liquid, from which the unsaponifiable material had been removed by means of ether, as above described, was acidified with sulphuric acid and distilled in a current of steam for ten hours. The combined distillates were then extracted twice with ether, after which the aqueous liquid was made alkaline with barium hydroxide, and evaporated to a low bulk. The excess of alkali was then removed by means of carbon dioxide, the filtered liquid further concentrated, and the acids present investigated by means of the silver salts. Having ascertained that considerable formic acid was present, this was first eliminated by heating the solution with silver nitrate until no further reduction occurred, when, after removing the precipitated silver, four fractions of colourless silver salt were obtained. These fractions, as well as

* Dried at 110°.

the similar ones subsequently referred to, were prepared by adding silver nitrate to a boiling solution of the salts of the volatile acids, when, on cooling the liquid, the silver salts of the organic acid separated in a well-crystallised condition. They were then collected, washed, dried in a vacuum over sulphuric acid, and analysed:

Fraction I. 0·5286 gave 0·2800 Ag. Ag = 53·0.
„ II. 0·3137 „ 0·1700 Ag. Ag = 54·2.
„ III. 0·4012 „ 0·2281 Ag. Ag = 56·9.
„ IV. 0·5091 „ 0·2979 Ag. Ag = 58·5.

$C_5H_9O_2Ag$ requires Ag = 51·7 per cent.
$C_4H_7O_2Ag$ „ Ag = 55·4 „
$C_2H_3O_2Ag$ „ Ag = 64·6 „

The above results appear to indicate that the volatile acids remaining dissolved in the distillate after extraction with ether consisted of formic, acetic, butyric, and valeric acids.

The ethereal extracts of the distillate were dried and the ether removed, when a quantity (55 grams) of liquid volatile acids was obtained. These acids were carefully fractionated five times under the ordinary pressure, employing a long-necked flask fitted with a rod and disk column, when the following fractions were obtained, all of which were optically inactive: (1) below 160°; (2) 160—170°; (3) 170—180°; (4) 180—190°; (5) 190—200°; (6) 200—210°; (7) 210—220°; (8) 220°+.

Fraction (1) amounted to 1·5 grams, and, since it contained some ether residues, was discarded.

Fraction (2) amounted to 6 grams. A portion of it was nearly neutralised with aqueous potassium hydroxide, and the silver salt prepared from it in four successive fractions. The first of these fractions became reduced owing to the presence of formic acid, but the remainder were analysed, with the following results:

Fraction II. 0·1564 gave 0·0812 Ag. Ag = 51·9.
„ III. 0·3665 „ 0·1895 Ag. Ag = 51·7.
„ IV. 0·3908 „ 0·2126 Ag. Ag = 54·4.

A remaining portion of the fraction IV of silver salt was fractionally crystallised, and the more soluble fraction analysed:

0·4495 gave 0·2474 Ag. Ag = 55·0.

$C_5H_9O_2Ag$ requires Ag = 51·7 per cent.
$C_4H_7O_2Ag$ „ Ag = 55·4 „

It thus appears that fraction (2) of the acids consisted essentially of valeric and butyric acids, and since it yielded a calcium salt which was more readily soluble in hot than in cold water, it is evident that the butyric acid present was the *iso*-form.

Fraction (3) amounted to 16 grams. A portion of it was

fractionally converted into the silver salt in the manner previously described, and the resulting 8 fractions analysed:

Fraction	I.	0·5424 * gave	0·2826 Ag.	Ag = 52·1.
„	II.	0·6544 „	0·3370 Ag.	Ag = 51·5.
„	III.	0·5709 „	0·2940 Ag.	Ag = 51·5.
„	IV.	0·4716 „	0·2429 Ag.	Ag = 51·5.
„	V.	0·7109 „	0·3673 Ag.	Ag = 51·7.
„	VI.	0·2867 „	0·1488 Ag.	Ag = 51·9.
„	VII.	0·7032 „	0·3783 Ag.	Ag = 53·8.
„	VIII.	0·7065 „	0·3798 Ag.	Ag = 53·8.

These results indicate that fraction (3) of the acids consisted chiefly of valeric acid, together with a little butyric acid. A direct analysis of the fraction gave the following result:

0·1229 gave 0·2588 CO_2 and 0·1068 H_2O. C = 57·4; H = 9·7.
$C_5H_{10}O_2$ requires C = 58·8; H = 9·8 per cent.
$C_4H_8O_2$ „ C = 54·5; H = 9·1 „

Fractions (4) and (5) amounted to 4 and 2 grams respectively, and, as they obviously consisted of mixtures of valeric acid and the constituent of fraction (6), they were not further investigated.

Isolation of a Hexenoic Acid, $C_6H_{10}O_2$.

Fraction (6) of the volatile acids amounted to 20 grams, thus representing more than one-third of the total mixture. It appeared to consist for the most part of an unsaturated substance, since, when dissolved in aqueous alkali, it instantly decolorised a solution of potassium permanganate:

0·1431 gave 0·3279 CO_2 and 0·1168 H_2O. C = 62·5; H = 9·1.
$C_6H_{10}O_2$ requires C = 63·2; H = 8·8 per cent.
$C_5H_{10}O_2$ „ C = 58·8; H = 9·8 „

Fraction (6) therefore appeared to consist of a hexenoic acid, contaminated with a very little valeric acid. It was refractionated twice under the ordinary pressure, the following fractions being collected: below 204°; 204—208°; 208°+.

The fraction boiling at 204—208° had D_{15}^{15} 0·9833, and was found to consist of a pure hexenoic acid:

0·1150 gave 0·2666 CO_2 and 0·0940 H_2O. C = 63·2; H = 9·1.
$C_6H_{10}O_2$ requires C = 63·2; H = 8·8 per cent.

The silver salt was somewhat sparingly soluble in boiling water, from which it separated, on cooling, in small, colourless leaflets:

0·5854 gave 0·2846 Ag. Ag = 48·6.
$C_6H_9O_2Ag$ requires Ag = 48·8 per cent.

* This fraction became slightly reduced.

Fraction (7) consisted also of the hexenoic acid, and amounted to 1·5 grams, whilst fraction (8) was so small as to be negligible.

Constitution of the Hexenoic Acid.

In order to ascertain which of the isomeric hexenoic acids the one occurring in hops represented, it was necessary to determine its constitution. A further quantity of the fraction of volatile acids boiling at 200—210° was therefore obtained by working up more of the original petroleum extract of the resin.

A quantity (28 grams) of this approximately pure hexenoic acid was dissolved in an excess of aqueous potassium hydroxide, and gradually treated in the cold with aqueous potassium permanganate until the colour of the latter was only slowly discharged, an amount of oxygen corresponding with a little less than two atomic proportions being thus consumed. The precipitated manganese dioxide was then removed by means of sulphur dioxide, and the clear liquid acidified and distilled in a current of steam for twelve hours. The distillate was rendered alkaline with potassium hydroxide, concentrated to a small bulk, acidified, and then extracted once with ether. This removed about 3 grams of a liquid which was found to consist chiefly of valeric acid, and had doubtless been originally contained in the fraction of acid oxidised. The aqueous liquid from which the valeric acid had been extracted was then distilled with steam, and the acids contained in the distillate converted into the barium salt, from which the silver salt was prepared in three fractions. The first of these fractions was small, and was found to consist of a mixture of silver butyrate and valerate, the former predominating. The second and third fractions, however, which represented by far the greater part of the volatile acid, consisted of silver butyrate (Found, Ag = 55·3. Calc., Ag = 55·4 per cent.). A quantity of this silver salt was converted into the corresponding calcium salt, which crystallised in tufts of needles, and was much more soluble in hot than in cold water, thus proving it to be calcium *iso*butyrate. It is evident, therefore, that *iso*butyric acid is a product of the oxidation of the hexenoic acid, since not more than a trace of it could have been present in the original fraction of acid boiling at 200—210°.

The original acid liquid remaining after the removal of the above-mentioned volatile acids by distillation with steam, and containing the non-volatile products of the oxidation, was concentrated to a small bulk, and then extracted thirty times with ether. This treatment removed a quantity (about 4 grams) of a solid product which, on fractional crystallisation from a mixture of ethyl acetate and benzene, was found to consist of oxalic acid, together with

another compound, the former predominating. The mixture was therefore converted into the calcium salt, and the calcium oxalate removed and analysed (Found, CaO = 43·6. Calc., CaO = 43·8 per cent.). The filtrate from the calcium oxalate was acidified, and extracted many times with ether, when, on removal of the solvent, a small amount of a crystalline acid was obtained. The latter, on recrystallisation from benzene containing a little ethyl acetate, separated in colourless leaflets, melting at 109°:

0·0787 * gave 0·1404 CO_2 and 0·0586 H_2O. C = 48·6; H = 8·3.
$C_6H_{12}O_4$ requires C = 48·6; H = 8·1 per cent.

This acid was thus identified as $\alpha\beta$-dihydroxy*iso*hexoic acid, $CH_3{\cdot}CH(CH_3){\cdot}CH(OH){\cdot}CH(OH){\cdot}CO_2H$, which has been described by Braun (*Monatsh.*, 1896, **17**, 216) as melting at 108°. On oxidation with chromic acid, it yielded *iso*butyric acid.

From the results of the above-described oxidation, it is evident that the hexenoic acid contained in hops is *β-iso*propylacrylic acid, $CH_3{\cdot}CH(CH_3){\cdot}CH{:}CH{\cdot}CO_2H$, a substance which has not previously been known to occur in nature. It has an odour resembling that of valeric acid, but somewhat more rank.

Isolation of a Phytosterolin, $C_{33}H_{56}O_6$.

The original alkaline solution obtained by the hydrolysis of the petroleum extract of the resin, and from which the volatile acids had been removed, as above described, was shaken with a large volume of ether, when a relatively small quantity of a sparingly soluble solid remained undissolved. This was collected, washed with ether, and then extracted in a Soxhlet apparatus, first with ethyl acetate until the chlorophyll was removed, and subsequently for a long time with alcohol. The solid which gradually separated from the latter solvent during the process of extraction was collected, and heated with acetic anhydride containing a little pyridine, when, on removing the greater part of the solvent, a product was obtained which crystallised from alcohol in colourless leaflets. This was hydrolysed with alcoholic potassium hydroxide, when it yielded a substance which separated from dilute pyridine in colourless, microscopic crystals, melting at 285—290°:

0·1147 gave 0·3039 CO_2 and 0·1084 H_2O. C = 72·3; H = 10·5.
$C_{33}H_{56}O_6$ requires C = 72·3; H = 10·2 per cent.

0·1918, made up to 20 c.c. with pyridine, gave α_D −0°37′ in a 2-dcm. tube, whence $[\alpha]_D$ −32·1°.

This substance was thus identified as a phytosterolin, or phytosterol glucoside (compare Power and Salway, this vol., p. 399).

* Dried at 90°.

The acetyl derivative melted at 167—168°, and was analysed:

0·1049 gave 0·2648 CO_2 and 0·0862 H_2O. C = 68·9; H = 9·1.
$C_{33}H_{52}O_6(CO\cdot CH_3)_4$ requires C = 68·7; H = 8·9 per cent.

The benzoyl derivative, prepared by benzoylation in pyridine solution, crystallised from a mixture of chloroform and alcohol in slender, colourless needles, melting at 198°:

0·0959 gave 0·2673 CO_2 and 0·0667 H_2O. C = 76·0; H = 7·7.
$C_{33}H_{52}O_6(CO\cdot C_6H_5)_4$ requires C = 75·9; H = 7·5 per cent.

*Half a gram of the phytosterolin was hydrolysed in amyl alcohol solution by heating with aqueous hydrogen chloride, together with sufficient ethyl alcohol to render the mixture homogeneous, when it yielded dextrose together with a phytosterol. The latter formed glistening leaflets, melting at 136°:

0·1041 gave 0·3204 CO_2 and 0·1127 H_2O. C = 83·9; H = 12·0.
$C_{27}H_{46}O$ requires C = 83·9; H = 11·9 per cent.

Examination of the Non-volatile Fatty Acids.

The ethereal solution from which the crude phytosterolin had been removed by filtration was evaporated, when a very dark green, viscid product was obtained, representing the greater portion of the original petroleum extract of the resin. It was dissolved in methyl alcohol, esterified by means of sulphuric acid, and the resulting product extracted with ether. The ethereal solution was then shaken with a large volume of aqueous 25 per cent. potassium hydroxide, after which the mixture separated into three layers. The lowest layer was an orange-coloured, aqueous liquid, and, when separated and acidified, yielded about 10 grams of a viscid, orange-coloured product, which possessed a somewhat bitter taste, but afforded nothing definite. The middle layer consisted of an almost black, viscid tar, and was found to consist of the potassium compound of a phenolic resin. The latter, when isolated, formed a dark greenish-brown resin, which amounted to about one-third of the total product; it was tasteless, and nothing definite could be obtained from it. The ethereal layer, on evaporation, yielded a dark green oil, consisting of the methyl esters of the fatty acids contaminated with chlorophyll. It was deprived of the latter by distillation under diminished pressure, after which the distillate was hydrolysed, the resulting fatty acids extracted with ether, and separated into their saturated and unsaturated components by means of their lead salts, in the usual manner.

* This experiment was conducted by Dr. Salway.

The Saturated Fatty Acids.

The acids obtained from the insoluble lead salts were esterified by means of methyl alcohol and sulphuric acid, and the resulting esters carefully fractionated five times under a pressure of 20 mm., with the employment of a long-necked flask, when the following fractions were collected: (1) below 205°; (2) 205—210°; (3) 210—215°; (4) 215—220°; (5) 220—225°; (6) 225—235°; (7) above 235°/20 mm.

Fractions (1) and (2) solidified on cooling, and represented the greater part of the material. They both consisted of practically pure methyl palmitate, and, on hydrolysis, yielded palmitic acid, which, after crystallisation, melted at 62·5° (Found, C=74·8; H=12·5. Calc., C=75·0; H=12·5 per cent.).

Fraction (3) was hydrolysed, but the resulting acid, even after very many crystallisations, did not appear homogeneous, and melted at 61—64·5°. It appeared to consist of a mixture of palmitic and stearic acids in about equal proportions (Found, C=75·6; H=12·6. Calc. for $C_{16}H_{32}O_2$, C=75·0; H=12·5; for $C_{18}H_{36}O_2$, C=76·1; H=12·6 per cent.).

Fraction (4) was small in amount, and, on hydrolysis, yielded an acid which, after crystallisation, melted at 66—67°, and was nearly pure stearic acid (Found, C=76·2; H=12·7. Calc., C=76·1; H=12·6 per cent.).

Fraction (5) was also small in amount. On hydrolysis, it yielded an acid which, when repeatedly crystallised from various solvents, appeared perfectly homogeneous. It formed glistening plates, melting constantly at 62·5—63°:

0·1098 gave 0·3088 CO_2 and 0·1272 H_2O. C=76·7; H=12·9.
$C_{20}H_{40}O_2$ requires C=76·9; H=12·8 per cent.

This acid is thus seen to agree in composition with arachidic acid, the melting point of which, however, has been recorded as 77°. It therefore appears probable that it is an isomeride of arachidic acid, and in that case would be a new compound. The results given above were confirmed by working up a further and larger amount of the original petroleum extract of the resin, but the total amount of the respective acid obtained was not sufficient conclusively to establish its homogeneity, although it had all the characters of a pure substance.

Isolation of Cluytinic Acid, $C_{21}H_{42}O_2$.

Fraction (6) was larger in amount than either of the two fractions immediately preceding it. It was hydrolysed, and the resulting

acid crystallised several times, when it formed lustrous leaflets, melting constantly at 69°:

0·0996 gave 0·2826 CO_2 and 0·1176 H_2O. C=77·3; H=12·9.

$C_{21}H_{42}O_2$ requires C=77·3; H=12·9 per cent.

This acid thus appeared to be identical with cluytinic acid, a compound which was only recently obtained by Tutin and Clewer from the South African plant, *Cluytia similis* (T., 1912, **101**, 2226). In order to exclude any possibility of doubt regarding its homogeneity, it was compared with all possible mixtures of higher and lower fatty acids having the same percentage composition, but the latter products were all of lower and indefinite melting point. Further evidence of the identity of the substance with cluytinic acid was afforded by working up an additional quantity of the original petroleum extract of the resin, when an acid was obtained which possessed the same composition and properties as that above described. Moreover, a quantity of the acid was converted into its methyl ester, and the latter crystallised several times from alcohol, when it was found to be homogeneous.

Methyl Cluytinate, $C_{20}H_{41}\cdot CO_2\cdot CH_3$.—This compound forms small, colourless, lustrous leaflets, melting sharply at 47°:

0·0930 gave 0·2640 CO_2 and 0·1087 H_2O. C=77·4; H=13·0.

$C_{22}H_{44}O_2$ requires C=77·6; H=12·9 per cent.

Isolation of Cerotic Acid.

Fraction (7) was a solid of appreciably higher melting point than any of the preceding fractions. It was crystallised successively from ethyl acetate and alcohol, when it separated in small, colourless leaflets, melting sharply at 58°, and evidently consisted of methyl cerotate. On hydrolysis, it yielded cerotic acid, which crystallised in small, colourless plates, melting at 79° (Found, C=78·7; H=13·2. Calc., C=79·0; H=13·2 per cent.).

The Unsaturated Fatty Acids.

The unsaturated fatty acids were regenerated from the soluble lead salts, and extracted with ether. They represented the largest individual product obtained from the hop extract, being about three times as great in amount as the saturated acids.

A portion of the material was converted into the methyl ester, and the latter fractionally distilled six times under 20 mm. pressure, with the employment of a long-necked flask, when the following fractions were collected, and in the amounts indicated: I, below 215° (9·7 grams); II, 215—218° (29·5 grams); III, 218—222° (94·1 grams); IV, 222—227°/20 mm. (4·5 grams). These fractions

were analysed, and their iodine values determined, with the following results:

Fraction	I.	C=76·1; H=11·6.	Iodine value=150·6.
„	II.	C=77·3; H=11·5.	Iodine „ =172·2.
„	III.	C=77·2; H=11·5.	Iodine „ =177·3.
„	IV.	C=77·1; H=11·4.	Iodine „ =164·4.

Methyl linolate, $C_{19}H_{34}O_2$, requires C=77·5; H=11·5 per cent. I.V.=172·7.

It thus appears that the unsaturated acids consisted almost entirely of linolic acid. The somewhat low figures obtained for the first fraction of esters was probably due to the presence of a little methyl palmitate.

Ether Extract of the Resin.

This extract, when free from solvent, formed a dark green, friable mass. A portion of the ethereal solution of the extract, representing 170 grams of dry material, was largely diluted with ether, when a quantity (about 18 grams) of a dark brown solid was precipitated. The latter was found to consist only of brown resin, together with a little phytosterolin. The filtrate was shaken with aqueous ammonium carbonate, and the alkaline liquid separated and acidified. A quantity of green, tarry material was thus obtained, which had a very bitter, nauseous taste, and an odour resembling that of valeric acid. As nothing definite could be directly separated from it, it was heated with alcoholic potassium hydroxide for some time, the alcohol then removed, water added, and the mixture acidified and distilled with steam. Nothing definite could be isolated from the contents of the distillation flask, but the distillate yielded about 2 grams of volatile acid. The latter distilled from 110° to about 245° under the ordinary pressure, and, after removing a little formic acid, the silver salt was prepared from it in three fractions. A small proportion of an unsaturated acid was present, which was doubtless the previously described hexenoic acid:

Fraction	I.	0·1335 gave	0·0543 Ag.	Ag=40·7.
„	II.	0·4597 „	0·2321 Ag.	Ag=50·5.
„	III.	0·2453 „	0·1245 Ag.	Ag=50·8.

$C_9H_{17}O_2Ag$	requires	Ag=40·7 per cent.
$C_6H_{11}O_2Ag$	„	Ag=48·4 „
$C_6H_9O_2Ag$	„	Ag=48·8 „
$C_5H_9O_2Ag$	„	Ag=51·7 „

The mixture of volatile acids obtained from the ether extract of

the resin therefore appears to have contained nonoic, hexenoic, and valeric acids, but other acids may also have been present.

The ethereal liquid which had been extracted with ammonium carbonate, as above described, was washed with water, when some chlorophyll was removed, and subsequently shaken with a considerable volume of fairly concentrated aqueous sodium carbonate. The mixture then separated into three layers, and the lowest layer, which was a deep yellow, aqueous liquid, was separated and acidified as quickly as possible. The mixture remaining in the separator, which consisted of a green, ethereal liquid, together with a large quantity of a black, tarry sodium compound of a resin beneath it, was then extracted many times with aqueous sodium carbonate, the alkaline liquids being in each case acidified as soon as possible.

Isolation of a New Phenolic Substance, Humulol, $C_{17}H_{18}O_4$.

The combined, acidified sodium carbonate extracts above mentioned were extracted with ether, and the ethereal solution fractionally extracted with alkalis in order to effect a more exact separation of its constituents. On shaking with ammonium carbonate, a quantity of a brownish-yellow resin was removed, and, since the original material had been deprived of substances soluble in this alkali, it must have been formed by the action of the sodium carbonate on some constituent originally present. It possessed a somewhat bitter taste, but nothing could be isolated from it. The ethereal solution was then shaken with a small quantity of very dilute, aqueous sodium carbonate, and subsequently washed with water. This treatment removed some green, resinous material and a quantity of chlorophyll. The ethereal liquid was subsequently extracted with many successive portions of concentrated aqueous sodium carbonate, the resulting alkaline extracts being quickly separated and acidified, when they were separately examined. The first three extractions yielded a product which, when isolated by means of ether and the resulting ethereal solution concentrated, deposited a quantity of a crystalline substance. The total amount of this substance obtained in the present instance and from the remaining quantity of ether extract of the resin was altogether about 5·6 grams, being thus equivalent to 0·0224 per cent. of the weight of hops employed. The crystalline substance was collected on a filter, and washed with ether, after which it was recrystallised by concentrating its solution in ether containing a little alcohol. As thus obtained, it formed small, almost colourless needles, which, when dissolved in alcohol, possessed a bitter taste. Various separately isolated portions of this substance

exhibited different behaviour on heating. Thus, most of them melted at about 150—154°, with the evolution of gas, whilst others only sintered at this temperature, fusion occurring at about 194° All the different quantities of bitter substance obtained gradually became yellow, however, when heated at 90°, after which they melted at about 140°, and when heated for half an hour at 135° they appeared to decompose, being converted into an orange-coloured resin and suffering a diminution in weight. It was found, however, that this behaviour was due to the presence of varying amounts of ether of crystallisation, since when the substance was recrystallised from 50 per cent. acetic acid, it formed small, pale fawn-coloured needles, which, when heated fairly quickly, melted at 196°, and did not change at 135°. On again recrystallising from ether, the product of low melting point was regenerated. A very careful examination of the substance proved it to be quite homogeneous, and, after crystallisation from dilute acetic acid, it was analysed:

0·1105 * gave 0·2880 CO_2 and 0·0634 H_2O. C = 71·1; H = 6·4.
0·1144 * „ 0·2972 CO_2 „ 0·0653 H_2O. C = 70·9; H = 6·3.
0·3550, in 29·02 of acetic acid, gave Δt −0·175°, whence M.W. = 273.
$C_{17}H_{18}O_4$ requires C = 71·3; H = 6·3 per cent. M.W. = 286.

It is evident from these results that the above-described substance possesses the formula $C_{17}H_{18}O_4$. As it does not agree with any substance of this formula hitherto described it is undoubtedly a new compound, and, with reference to its phenolic properties and its source, it is proposed to designate it *humulol*.

Humulol dissolves in aqueous sodium carbonate, but not in ammonium carbonate or hydroxide. Its solution in alkali is pale yellow, but becomes deep yellow when kept for some time, or when warmed. It dissolves in concentrated sulphuric acid, yielding a pale yellow liquid, and, in alcoholic solution, gives no coloration with ferric chloride. It is insoluble in water, light petroleum, or benzene, very sparingly soluble in ether or chloroform, moderately so in glacial acetic acid, and very readily so in alcohol or pyridine. On acetylation, it yielded a colourless product, which, however, was a viscid, uncrystallisable liquid.

Hydrolysis of Humulol: Formation of p-*Hydroxybenzaldehyde.*

About 3 grams of humulol were dissolved in 20 per cent. aqueous potassium hydroxide, and the solution boiled for half an hour. The liquid first became deep yellow, but subsequently much lighter in colour. It was acidified, and extracted several times with ether,

* Dried at 135°.

the ethereal liquid being then extracted successively with aqueous ammonium carbonate, sodium carbonate, and potassium hydroxide.

The alkaline liquid obtained by extraction with ammonium carbonate yielded, on acidification and extraction with ether, a small amount of a crystalline product, which evidently was an acid. This was recrystallised from benzene, when it was obtained in small, nearly colourless prisms, melting at 210°:

0·0697 gave 0·1676 CO_2 and 0·0324 H_2O. C = 65·6; H = 5·2.

$C_{15}H_{14}O_5$ requires C = 65·7; H = 5·1 per cent.

This acid would thus appear to possess the formula $C_{15}H_{14}O_5$, and in that case would be a new compound. The amount obtained, however, was not sufficient for its further examination.

The sodium carbonate extract yielded, on acidification and extraction with ether, a larger amount of material. It was crystallised first from benzene and subsequently from water, when colourless needles were obtained, melting at 118°:

0·0860 gave 0·2168 CO_2 and 0·0393 H_2O. C = 68·8; H = 5·1.

$C_7H_6O_2$ requires C = 68·9; H = 4·9 per cent.

This substance yielded a dark violet colour with ferric chloride, and was identified as *p*-hydroxybenzaldehyde. When mixed with an authentic specimen of the latter compound, the melting point remained unchanged.

The potassium hydroxide extract of the ethereal solution of the hydrolytic products yielded, on extraction with ether, a pale brown, viscid product, which could not be crystallised, and gave no coloration with ferric chloride. It yielded an oily acetyl derivative, and when methylated by means of methyl sulphate and potassium hydroxide, a similar oily product was obtained. On distilling the latter under diminished pressure, however, it was found not to be homogeneous, but the amount available did not permit of its further investigation.

Isolation of a New Yellow Phenolic Substance, Xanthohumol, $C_{13}H_{14}O_3$.

As previously mentioned, the above-described substance, humulol, was isolated from the liquids obtained by the first three extractions with concentrated aqueous sodium carbonate. Most of the subsequently obtained sodium carbonate extracts, however, also yielded small amounts of humulol, which was readily separated on account of its sparing solubility in pure ether. On examining the filtrates from these small residual amounts of humulol, it was found that they contained a second substance, which was only very slowly removed from its solution in ether by fairly concentrated aqueous sodium carbonate, but much more readily by potassium hydroxide.

The total amount of material readily soluble in pure ether, obtained from all but the first three extractions with aqueous sodium carbonate, was brought together, and the ether removed. A small amount of alcohol was then added to the green, viscid residue, and the mixture kept for some days, when crystallisation gradually ensued. The green, viscid mixture containing the crystalline solid was then drained on a porous tile in an atmosphere saturated with alcohol, when a deep orange-coloured, crystalline substance was obtained. The latter was crystallised from a small volume of alcohol, when it formed glistening, orange-coloured, acicular crystals, melting at 169·5°:

0·1071 * gave 0·2790 CO_2 and 0·0613 H_2O. C=71·0; H=6·4.

An additional amount of this compound was obtained in a similar manner from a further quantity of the original ether extract of the resin, and was crystallised three times from alcohol. It then melted constantly at 172°:

0·0855 gave 0·2239 CO_2 and 0·0495 H_2O. C=71·4; H=6·4.
$C_{13}H_{14}O_3$ requires C=71·5; H=6·4 per cent. M.W.=218.

A determination of the molecular weight of the substance by Barger's microscopic method was kindly made for us by Mr. A. J. Ewins, with the following result:

0·0554, in 1·172 of pyridine, using benzil as the standard, was between 0·225 and 0·250 mol. Mean M.W.=184.

It would appear from the above results that the orange-coloured substance possesses the formula $C_{13}H_{14}O_3$. Since it differs in its properties from any known substance of this composition, it is evidently a new compound, and, with consideration of its characters and its source, it is proposed to designate it *xanthohumol*.

Xanthohumol, although possessing practically the same percentage composition as the previously described compound, humulol, is not an isomeride of the latter, as is shown by the determination of their respective molecular weights. The two compounds are, moreover, quite different in their properties. Thus, xanthohumol is devoid of bitter taste, and is not decomposed by heating with alkalis, but dissolves in the latter with an intense yellow colour. In alcoholic solution it gives no coloration with ferric chloride. When dissolved in sulphuric acid it yields at first a very deep orange-coloured liquid, which, however, soon becomes nearly colourless. It is very readily soluble in ether, ethyl acetate, or pyridine, moderately so in alcohol, and rather sparingly so in benzene or glacial acetic acid. On acetylation or methylation, it yielded only

* Dried at 135°.

viscid, uncrystallisable oils. The total amount of xanthohumol obtained was about 2 grams, being thus equivalent to about 0·008 per cent. of the weight of hops employed.

The alcoholic mother liquors from the first recrystallisation of the xanthohumol, when slowly evaporated, were found to contain a small amount of yet another compound. The latter formed very small, pale yellow needles, but the amount present was not sufficient to permit of its further characterisation.

The original ethereal solution of the ether extract of the resin, which had been extracted many times with aqueous sodium carbonate, as previously described, was separated from the large amount of black, tarry material which had been produced during the extractions with alkali. This dark-coloured material, which possessed a somewhat bitter taste, was entirely resinous in character, and nothing could be isolated from it. The ethereal solution, however, on extraction with aqueous potassium hydroxide, yielded a further small amount of the previously described humulol, together with amorphous products. The final ethereal solution of the neutral constituents of the resin was evaporated, and the residue, from which nothing definite could be directly separated, was heated with alcoholic potassium hydroxide. This treatment, however, led only to the isolation of a further small amount of the previously mentioned phytosterolin, together with chlorophyll and amorphous products.

Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resin.

The chloroform, ethyl acetate, and alcohol extracts of the resin were all amorphous solids, which, when dissolved in alcohol, possessed a somewhat bitter taste. The chloroform extract had a dull green colour, but the last-mentioned two extracts were dark brown. All of these extracts were examined, but nothing definite could be isolated from them, and, on heating with dilute sulphuric acid in aqueous alcohol, they were found to contain nothing glucosidic.

Summary and Conclusions.

The material used in this investigation consisted of a good quality of fresh Kentish hops of the crop of 1911.

The air-dried material contained 10·48 per cent. of moisture, 2·4 per cent. of tannin, and, on incineration, yielded 7·919 per cent. of ash.

For the purpose of a complete examination, 25 kilograms of hops were employed. This material was ground, completely extracted

with hot alcohol, and the resulting extract distilled in a current of steam in order to remove the essential oil.

From the portion of the extract which was soluble in water there were isolated small amounts of choline, $C_5H_{15}O_2N$, and of *l*-asparagine, $C_4H_8O_3N_2$, both of which had previously been observed to be present, although no evidence of the identity of the last-mentioned substance appears to have hitherto been recorded. The aqueous liquid also contained, besides tannin, a quantity of potassium nitrate and a sugar which yielded *d*-phenylglucosazone (m. p. 208°), together with dark-coloured, amorphous material which possessed an intensely bitter taste. A volatile base, having a coniine-like odour, was also obtained, but the amount was so extremely small that it could not be further characterised.

The portion of the alcoholic extract which was insoluble in water consisted of a dark green, oily resin, the amount of which was equivalent to about 14·1 per cent. of the weight of hops employed. From this resin, which still contained some occluded essential oil, the following compounds were isolated: (i) ceryl alcohol, $C_{27}H_{56}O$; (ii) hentriacontane, $C_{31}H_{64}$; (iii) a phytosterol, $C_{27}H_{46}O$; (iv) a phytosterolin (phytosterol glucoside), $C_{33}H_{56}O_6$; (v) a mixture of volatile fatty acids, consisting of formic, acetic, butyric, and valeric acids, together with a hexenoic acid, $C_6H_{10}O_2$ (b. p. 204—208°), which was identified as *β-iso*propylacrylic acid, and apparently a little nonoic acid, $C_9H_{18}O_2$, was also present; (vi) saturated and unsaturated non-volatile acids, comprising: palmitic, stearic, and cerotic acids, and an acid, $C_{20}H_{40}O_2$ (m. p. 62·5—63°), which is apparently an isomeride of arachidic acid; furthermore, cluytinic acid, $C_{21}H_{42}O_2$ (m. p. 69°), the *methyl* ester of which melts at 47°, and linolic acid; (vii) a new, crystalline, phenolic substance, *humulol*, $C_{17}H_{18}O_4$ (m. p. 196°), possessing a pale fawn colour and a bitter taste, which, on hydrolysis with potassium hydroxide, yielded, among other products, an *acid*, $C_{15}H_{14}O_5$ (m. p. 210°), and *p*-hydroxybenzaldehyde; (viii) a new, crystalline, phenolic substance, *xanthohumol*, $C_{13}H_{14}O_3$ (m. p. 172°), which is tasteless and possesses an orange-yellow colour. The statements made many years ago that hops contain a yellow colouring matter resembling quercitrin, and also a wax consisting of myricyl palmitate, cannot be confirmed.

A consideration of the results of the present investigation leads to the following conclusions. In the first place, the bitterness of hops is not due to any single substance, such as the so-called "hop-bitter acid" or "lupulic acid," but is to be attributed to a number of products, most of which are amorphous. Some of these products are soluble in water, whilst others represent constituents of the

resin. One well-defined, crystalline substance, which possesses a bitter taste, has, however, now been isolated from the resin, and, as above noted, has been designated *humulol*. Furthermore, the differentiation of the resinous material as α-, β-, and γ-resins is misleading, inasmuch as this material is much more complex in character than would be indicated by the methods employed for the separation of the respective products.

It may finally be noted that, although various products have hitherto been obtained from hops which were supposed to represent the bitter principles, the characters ascribed to them renders it evident that they were of a very indefinite nature. On the other hand, all previous investigators appear completely to have overlooked the important fact that the resinous material contains a large proportion of the ordinary fatty acids and their esters. Such of the numerous methods proposed for the valuation of hops as are based on the titration of extracts obtained by means of light petroleum and similar solvents are therefore of very doubtful utility.

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